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# Modeling highly pathogenic avian influenza transmission in wild birds and poultry in West Bengal, India

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Wild birds are suspected to have played a role in highly pathogenic avian influenza (HPAI) H5N1 outbreaks in West Bengal. Cluster analysis showed that H5N1 was introduced in West Bengal at least 3 times between 2008 and 2010. We simulated the introduction of H5N1 by wild birds and their contact with poultry through a stochastic continuous-time mathematical model. Results showed that reducing contact between wild birds and domestic poultry, and increasing the culling rate of infected domestic poultry communities will reduce the probability of outbreaks. Poultry communities that shared habitat with wild birds or those indistricts with previous outbreaks were more likely to suffer an outbreak. These results indicate that wild birds can introduce HPAI to domestic poultry and that limiting their contact at shared habitats together with swift culling of infected domestic poultry can greatly reduce the likelihood of HPAI outbreaks.

Highly pathogenic avian influenza (HPAI) H5N1 (hereafter H5N1) first emerged in 1996 in Eastern Asia and has been circulating amongst avian populations since then. Today, H5N1 has spread into parts of Asia, Europe, Africa and the Middle East. The 2005 H5N1 outbreak in China's Qinghai Lake migratory birds resulted in more than 6000 bird mortalities<sup>1,2</sup>. Subsequent migratory wild bird mortalities were observed in the same year in Mongolia, Kazakhstan, western parts of China and Eastern Europe<sup>3,4</sup>. Migratory birds are known to carry the infection<sup>5</sup> which supports the hypothesis they may have played a role in the spread of H5N1<sup>6</sup>. However, some believe that it is unlikely for a bird to carry such highly pathogenic virus during the physiologically taxing activity of migration<sup>7</sup>. In contrast, the spread of H5N1 along wild bird migratory routes may be explained by recent studies suggesting that previous exposure of birds to low pathogenic avian influenza may provide resistance to H5N1<sup>8</sup>. Furthermore, it is also suggested that there might be instances where the virus is transmitted in cycles of infections in bird flocks during migration<sup>9</sup>. A global study investigating sources of H5N1 infections in poultry in 2006 indicated that 3 out of 21 outbreaks in Asia were introduced by migratory birds<sup>10</sup>.

India's first H5N1 outbreak was reported in January of 2006 in the Navapur District, Maharashtra, India. Since then H5N1 has been reported in 8 different states. In West Bengal, 54 H5N1 outbreaks in poultry were reported between Jan 2006 and Aug 2010 making it the state with highest incidence in India<sup>11</sup>. Very few studies have been conducted about the epidemiology of H5N1 outbreaks in India. Gilbert and others studied the epidemiological risk factors associated with the migrations of sentinel species in the area of West Bengal and adjacent Bangladesh<sup>9</sup>. Adhikari and others estimated the disease incidence using an ecological niche modeling tool<sup>12</sup>. Fewer large-scale monitoring studies have been conducted to estimate the prevalence of H5N1 in wild birds in India<sup>13,14</sup>. HPAI models created until now have considered a single introduction of virus from wild birds to poultry. Others concerning wild birds have included a continuous transmission model in wild birds exclusively<sup>15</sup>. Few mathematical models included both wild birds as well as poultry with continuous contact with each other<sup>16,17</sup>. To date, H5N1 outbreaks in India are restricted to poultry except for outbreaks in Jungle Crow (*Corvus macrorhynchos*) that took place in February 2012<sup>11</sup>. Models describing such outbreaks have focused on poultry dynamics<sup>18,19</sup>. Hence, modeling H5N1 outbreaks with continuous contact between wild birds and poultry along with studies focusing on risk factors related to wild birds in India are lacking.

Much work has been done to model bird migration and correlating the spatial and temporal migration pattern with the spread of H5N1. Many studies illustrated migration as stopovers<sup>4,20,21</sup>. A modeling study about disease



dynamics in bar-headed geese *Anser indicus* indicated these wild bird populations suffer greater mortality during the autumn versus spring migration due to the higher number of outbreaks in the autumn<sup>22</sup>. Bourrouiba et al (2011) also studied the interaction between poultry and migratory birds sustaining virus over long periods in China<sup>23</sup>. However, H5N1 viruses, if present, are thought to generally be at very low prevalence among migratory birds. Few samples have been collected from live birds in the region of this study<sup>24</sup>, and except for recent outbreaks in crows in India there are no reports of the virus in wild birds from India<sup>11</sup>. Thus studies of less than 10,000 birds are not likely to detect HPAI<sup>25</sup>. Larger studies estimate low prevalence (2.7%) of H5 subtypes elsewhere in the World<sup>26</sup>. Although the H5N1 virus is now endemic in the study region, it is speculated that the first introductions were because of migratory birds<sup>10</sup>. It is well documented that viruses do circulate in wild bird populations and that the virus populations grow at waterfowl wintering grounds. Thus it is reasonable to conclude in this study that first introductions of H5N1 were through migratory birds. The current study illustrates infection dynamics of H5N1 virus in wintering grounds of West-Bengal assuming that the first introduction of the virus is from a migratory bird.

The focus of this research was to describe the epidemiology of H5N1 outbreaks in West Bengal, India from 2008 to 2010. The first objective was to describe the regional epidemiology of H5N1, spatio-temporal clusters and transmissibility as estimated by the basic reproductive number. Second, a stochastic mathematical model was designed to investigate the role of wild bird congregations in the regional epidemiology of H5N1 in West Bengal, and a sensitivity analysis was performed to identify potentially effective control measures for H5N1 transmission. The model specifically illustrates the dynamics of the disease in poultry as well as wild bird congregations (which is a mixture of resident and wintering migratory birds), unlike previous models mentioned which simulate spread during migration. Finally, risk factors relating to wild migratory bird congregations and H5N1 outbreaks in poultry in West Bengal were investigated.

## Results

We conducted a series of analysis on the epidemiological data on H5N1 outbreaks in poultry in West Bengal. Here we present results for spatio-temporal cluster analysis, basic reproductive number estimation, stochastic modeling for disease dynamics in wild birds and poultry, and identify important risk factors related to wild birds for H5N1 outbreaks in West Bengal.

**Cluster analysis.** One most likely spatial cluster of 95.92 km radius ( $p < 0.01$ ) and two secondary spatial clusters were identified. Temporal cluster analysis showed that all the outbreaks under the study in the time frame of 1st January 2008 to 1st December 2010 were clustered into a single temporal cluster. The spatio-temporal cluster analysis also showed one most likely cluster and two secondary clusters. Location and time frame of spatio-temporal clusters are presented in Figure 1 and details of these clusters, time frames and relative risks are presented in Table 1.

**Basic Reproductive Number  $R_0$ .** Three  $R_0$  calculation methods were used and yielded very similar estimates. The formula for SI disease models described by Ward et al<sup>27</sup> estimated the mean of the transmission parameter  $\beta$  at 0.127 (Supplementary Table S1) with values that were exponentially distributed ( $\mu = 0.122$ ). The estimate for  $R_0$ , assuming a seven-day infectious period prior to culling, was  $0.859 (\pm 0.326 \text{ S.D.})$ . Doubling time of the outbreak was calculated to be 10 days from observed data and hence  $R_0$  was calculated to be 1.069. The mean number of times an outbreak was source to a future outbreak, based on the outbreak site being nearest neighbor in previous seven days, was  $0.969 (\pm 0.182 \text{ S.D.})$ . The estimate for  $R_0$

based on the simulated outbreak was 1.034. Descriptive statistics for the  $R_0$  estimates are presented in Table 2.

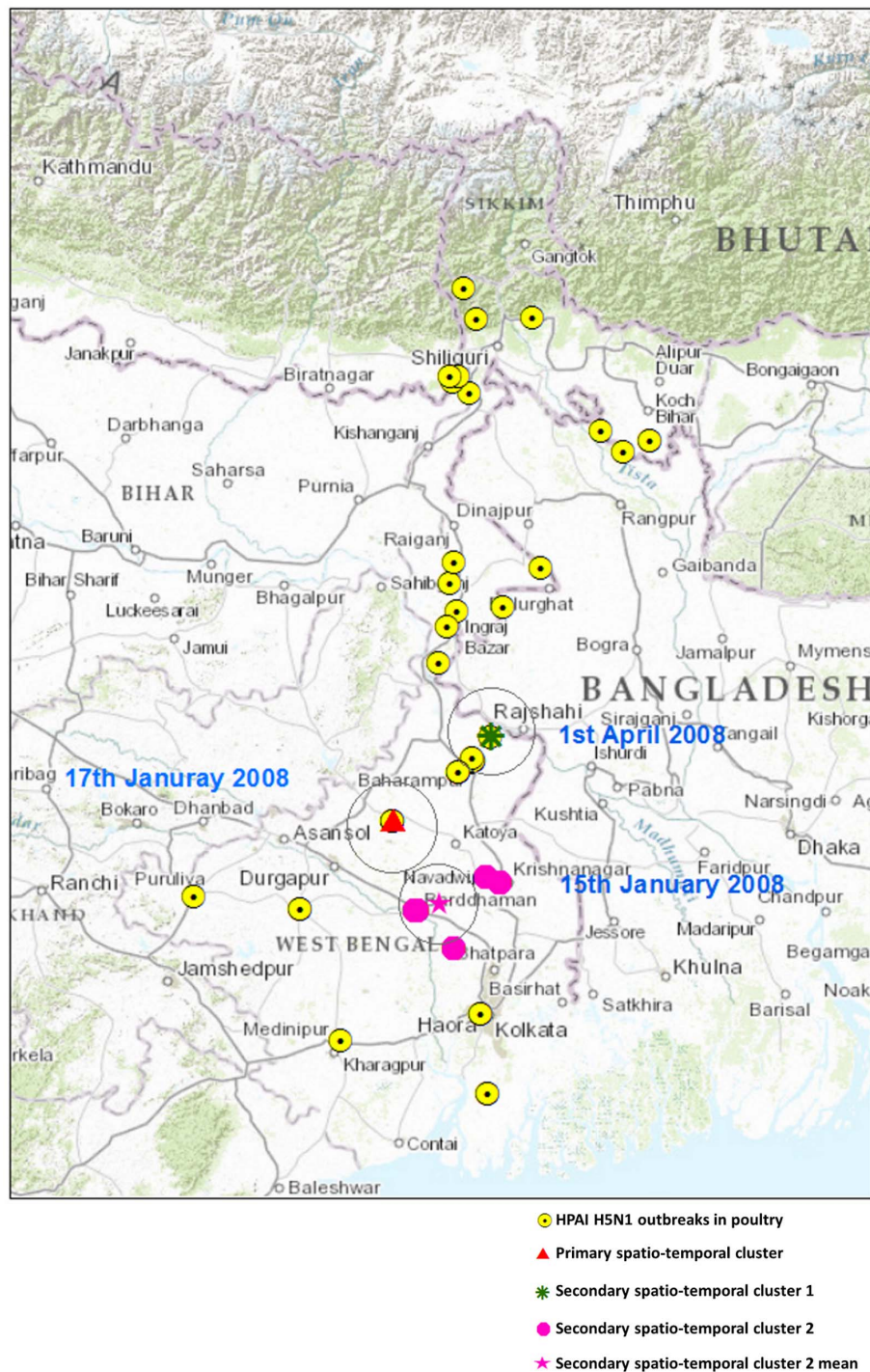
**Stochastic Model.** The mean time to maximum number of infected villages in 1000 iterations showed an epidemic curve with a peak at the 80<sup>th</sup> day of introduction of infection. On average 1.91% of villages suffered an outbreak during the study period while 98.5% of villages remained susceptible after the peak of the epidemic. Descriptive statistics of susceptible, infected and culled villages and peak time are presented in Table 3.

Sensitivity analysis for the model inputs showed that besides the transmission parameters for wild birds and poultry, the proportion of wild birds sharing the habitat with poultry ( $V$ ) and culling rate ( $C_u$ ) had the next greatest influence on H5N1 transmission. For  $\beta$ , the Spearman Rank correlation coefficient estimate with  $R_0$  was 0.90 and for number of villages infected at peak was 0.93. Reducing the  $\beta$  to half resulted in an increase in the probability of no outbreak from 0.93 to 0.99 and reduced the estimate for  $R_0$  from 1.034 to 1.00. When  $V$  was reduced systematically from 0.5 to 0.1 in decrements of 0.1,  $R_0$  decreased from 1.034 to 1.027 while the probability of no outbreak increased from 0.93 to 0.95. In addition, the number of villages infected at the end of the epidemic was reduced from 172 to 113 as  $V$  was changed from 0.5 to 0.1. When  $C_u$  was reduced from 7 to 3 days  $R_0$  changed from 1.0283 to 1.000, while the probability of no outbreak increased from 0.94 to 0.98. Combination of  $C_u = 0.3$  and  $V = 0.2$  resulted in a substantial increase in the probability of no outbreak to 0.99 and a reduction in  $R_0$  to 1.002. Spearman correlation coefficients are depicted in Figure 2.

**Logistic regression model.** Domestic ducks sharing the same habitat with wild birds and previous history of outbreak in the same district were the only two significant predictors for an H5N1 outbreak in poultry (Table 4). Previous history of outbreaks in the same district increased the odds of suffering outbreaks by 4.07 (95% confidence interval; 1.34–12.4). Model results were adjusted for the distance from a village's wild bird community which was found to be a confounder of the association between shared habitat and occurrence of an H5N1 outbreak.

## Discussion

The current study is the first epidemiological investigation of H5N1 outbreaks in West Bengal to apply spatial, statistical and mathematical modeling tools to investigate the role of wild birds in the spread of the disease in poultry. This is the first study to estimate  $R_0$  for outbreaks in West Bengal. In addition, this is the first study to compare wild bird related risk factors. Stochastic model results and conclusions from the risk factor analysis, both indicate that domestic poultry, especially domestic ducks, sharing habitat with wild bird congregations is an important risk factor in predicting H5N1 outbreaks in poultry. Mathematical modeling of H5N1 transmission showed that reducing contact between poultry ( $\beta$ ) remains the most important factor for controlling the spread of the disease. Sensitivity analysis of the stochastic model indicated that the mixing of domestic birds and wild birds was the most influential wild bird related variable influencing the probability of occurrence of an outbreak. The two most influential factors were poultry related. Previous models of avian influenza related to wild birds reported on the dynamics of the infection in the wild birds during migration, specifically from 'breeding ground' to 'wintering ground' and back to 'breeding ground'<sup>28,22</sup>. The current study focused on wintering grounds migration flux that occurs in the Indian subcontinent during the winter months from November to February. The virus is known to be carried over long distances from the northern breeding grounds circulating in the wild bird population (a mix of local resident wild birds and migratory wild birds) and poultry. Galworthy et al described a slight delay in the migration because of the persistent infection<sup>28</sup>. Pre-immunization of



**Figure 1** | Spatio-temporal clusters of Highly Pathogenic Avian Influenza H5N1 outbreaks in poultry in West Bengal, India during the period between 2008 to 2010 (Map used by permission. Copyright © Esri. All rights reserved).

wild birds with low pathogenic avian influenza is known to reduce the intensity of HPAI infection<sup>29</sup>, and is modeled in detail by Bourouiba et al<sup>23</sup>. Even though there are observations of low pathogenic viruses in wild birds in India<sup>14,24</sup> our model does not account for changes due to prior possible LPAI infection.

Risk factors analyzed in the study indicated that locations with constant interaction between wild birds and poultry may play an important role in transmission of HPAI from wild birds to poultry. Small ponds in every village specifically built for fishing by locals are generally considered the main sites where wild birds and domestic

ducks co-mingle. Rice-duck-human interactions are speculated to create ideal conditions to sustain HPAI, especially in the state of West Bengal<sup>30</sup>. A study conducted in Thailand also confirmed the role of domestic waterfowl in sustaining HPAI<sup>31</sup>.

Outbreaks of H5N1 in the West Bengal India between 2008 and 2010 were not randomly distributed in time and space. Spatio-temporal differences in outbreaks indicated that the H5N1 virus was introduced at least three times in the susceptible populations between 2008 and 2010. Phylogenetic studies done on virus isolates from these outbreaks also indicate that there have been multiple





**Table 1 | Observed spatial, temporal and spatio-temporal clusters of Highly Pathogenic Avian Influenza H5N1 outbreaks in poultry in West Bengal, India during the period 2008 to 2010**

Spatial Clusters							
	Nearest town (District)	Mean		No. of Locations	Radius (km)	Relative Risk	P value
		Latitude	Longitude				
<b>Most likely</b>	Bolpur-Santiniketan (Birbhum)	23.64825	87.70186	13	95.92	6.53	<0.001
<b>Secondary (1)</b>	Bhagwangola(Murshidabad)	24.319	88.377	1	0	10.86	<0.001
<b>Secondary (2)</b>	Dinhata(Cooch Behar)	26.14	89.4562	1	0	4.01	<0.001
Temporal Clusters							
	Nearest town (District)	Mean		No. of Locations	Time Frame	Relative Risk	P value
		Latitude	Longitude				
<b>Most likely</b>	Kandi (Murshidabad)	23.917347	88.026247	33	2008/1/1 to 2010/12/1	17.18	0.001
Spatio-Temporal Clusters							
	Nearest town (District)	Mean		No. of Locations	Time Frame (Radius km)	Relative Risk	P value
		Latitude	Longitude				
<b>Most likely</b>	Labpur(Birbhum)	23.8018	87.7133	1	2008/1/17 to 2008/1/17 (0)	104.29	<0.001
<b>Secondary (1)</b>	Bhagwangola(Murshidabad)	24.319	88.377	1	2008/4/1 to 2008/4/1 (0)	38.83	<0.001
<b>Secondary (2)</b>	Kuchut (Bardhaman)	23.26625	88.043465	10	2008/1/15 to 2008/1/15 (62.7)	13.29	<0.001

sources of the virus in the country during the early phase I outbreaks<sup>32</sup>. This study may be limited by possible under reporting of outbreaks due to the lack of formal reporting systems in remote villages, or underreporting by backyard poultry farmers because of fear of possible economic loss and restriction on poultry business. This explains the difference in observed and predicted number of infected villages. Underreporting may also explain the low  $R_0$  estimated in this study. However, reporting of backyard poultry deaths may have changed because of the compensation offered by government for submitting ailing birds for culling<sup>33</sup>. Government authorities responded to each outbreak by implementing depopulation, disinfection and serological surveillance of sentinel poultry birds. These interventions might have influenced distribution of the disease. Understanding the impact of these interventions is very difficult as the outcome in absence of these interventions is never observed<sup>34</sup>.

Estimates of  $R_0$  with nearest neighbor method, SI method and epidemic doubling time ranged from 0.859 to 1.069. The  $R_0$  estimates may inform decision makers whose responsibility is to devise control strategies. The variation of the  $R_0$  from 0.859 to 1.069 indicates there might be multiple primary sources for the outbreak and this should be further investigated<sup>35</sup>.  $R_0$  estimated using the SI model was considerably lower than 1 ( $0.859 \pm 0.328$  S.D.). However, the outbreak

did take place. This phenomenon may be explained by under reporting of infected villages as indicated in table S1 by several weeks of zero infected villages after the start of the epidemic (Table: S1) which in turn biased the mean value of  $R_0$  to below 1.

The estimates of  $R_0$  from the three methods used in this study vary considerably from previously reported estimates. Liu et al<sup>16</sup> estimated  $R_0$  to be 3.3 while Bouma et al<sup>36</sup> estimated it at 1.6 using mathematical models. While the H5N1 outbreak in Romania in 2006 had an estimated  $R_0$  ranging from 1.95 to 2.68 based on 161 infected villages<sup>27</sup>. The current study is based on only 33 outbreaks in West Bengal.

It is essential to estimate the  $R_0$  using different methods, because  $R_0$  depends on contact patterns between susceptible and infectious individuals or populations<sup>27</sup>. The methods used in estimation of  $R_0$  assume that a population of village poultry was infectious for 7 days. Other studies estimate the incubation period of the disease to be 2–6 days or sometimes 1–4 days<sup>37,38</sup>. However, studies which consider the village as its epidemiological unit consider 7 days as infectious period because accurate estimates of the period of infectiousness are unavailable<sup>27</sup>. A similar assumption was made where nearest infectious neighbor population was considered to be source of the new outbreak. While the real mechanism of virus spread might be due to movement of poultry or wild birds, through live markets, or fomites,

**Table 2 | Descriptive statistics for the basic reproductive number ( $R_0$ ) for Highly Pathogenic Avian Influenza H5N1 outbreaks in poultry in West Bengal, India during the period between 2008 to 2010 as estimated by 4 different methods**

Method of calculating $R_0$	$R_0$ Mean	Standard deviation	$R_0$ Median	Minimum	Maximum
<b>Nearest neighbor*</b>	0.970	0.182	1	0	4
<b>SI model**</b>	0.859	0.328	0	0	7.000
<b>Doubling time***</b>	1.069	-	-	-	-
<b>Stochastic mathematical model</b>	1.034	-	-	-	-

\*Nearest neighbor method of calculating  $R_0$  where nearest infectious village was considered as a source of a new outbreak.

\*\*In Susceptible Infected (SI) model method  $\beta$  (Transmission factor) was estimated and  $R_0$  was estimated using  $\beta I$ , where  $I$  is length of infectious period.

\*\*\*Epidemic doubling time was estimated from the observed outbreak and  $R_0$  was calculated using  $R_0 = 1 + (\ln(2)/t_0) * D$ , where  $t_0$  is the time for epidemic to double and  $D$  is mean incubation period.



**Table 3 | Descriptive Statistics for the number of villages with susceptible, infected and culled poultry flocks, and time at peak of outbreaks of Highly Pathogenic Avian Influenza H5N1 predicted by a continuous-time stochastic mathematical model**

Villages	Mean (%)	Standard deviation	Median (%)	Mode (%)
<b>Susceptible</b>	37378 (98.5)	4335	37945 (100)	1 (0.002)
<b>Infected</b>	266 (0.70)	2007	0 (0)	1 (0.002)
<b>Culled</b>	461 (1.21)	3760	0 (0)	1 (0.002)
<b>Peak time (days)</b>	23	18	11	51

the closest infectious village to each outbreak was assumed to be the source of infection<sup>39–41</sup>. Control strategies can be aimed at reducing  $R_0$  to less than one and hence reduce the critical coverage proportion of vaccination to achieve herd immunity in future prevention efforts.

Modeling results are useful in understanding the size of an HPAI outbreak which helps decision-makers to formulate control measures to avoid or control future outbreaks, something that was lacking after the 2008–2010 outbreaks<sup>33</sup>. The International Office of Epizootics states that places with large wetlands and high density of poultry in contact with wild birds can be a very important risk factor and prevention measures can be concentrated in such regions<sup>42</sup>. Prevention measures include reducing the contact between wild birds and domestic ducks by not allowing the domestic birds to forage freely near wetlands. Educational programs, targeting backyard flock owners should recommend reducing the contact between poultry and wild birds, reporting suspect cases and expedited culling to reduce the risk of HPAI transmission.

Wild birds carry avian influenza viruses from their breeding to wintering grounds. This study indicated that contact between wild birds and poultry is one risk factor for outbreaks. The state of West Bengal is a major rice producing state, producing two crops per year unlike other states that produce a single crop per year. In addition, West Bengal has large numbers of domestic ducks that interact with wild birds and poultry. These ducks generally graze and swim in the same habitat where migratory ducks and other wild birds congregate. HPAI virus can be easily transmitted at such places via fecal oral route. Control of the virus spread should be concentrated in water-bodies shared between wild birds and domestic ducks and poultry. Surveillance can be targeted on such water-bodies where wild birds and domestic ducks share habitat. Organized poultry farms need to increase their biosecurity to prevent outbreaks, including keeping

domestic ducks separate from other poultry and reducing contact between their poultry flocks and wild birds.

## Methods

**Data collection.** The National Institute of Virology and the Ela Foundation of India collected migratory bird data as part of an avian influenza surveillance project in 2010. The data included observations of known wintering grounds along with incidentally found congregations on large natural water-bodies, flooded rice fields, and reservoirs. Data available regarding avian influenza risk factors for these congregations included avian community size, habitat, sentinel species, species previously known positive for H5N1, bridge species, and occurrence of domestic ducks in the same water-body<sup>43–45</sup>. Observations were done using standard “point count” techniques by authors (SP and PP)<sup>46</sup>. Previous observations and ornithological records suggest that the observed risk factors at these wintering water-bodies remain constant from one migratory season to the next<sup>47,48</sup>. We used data on outbreaks which occurred during the years 2008 to 2010 in West Bengal (available from the OIE World Animal Health information Database (WAHID)). The data included reported bird mortalities, outbreak cases, and susceptible birds during the outbreaks.

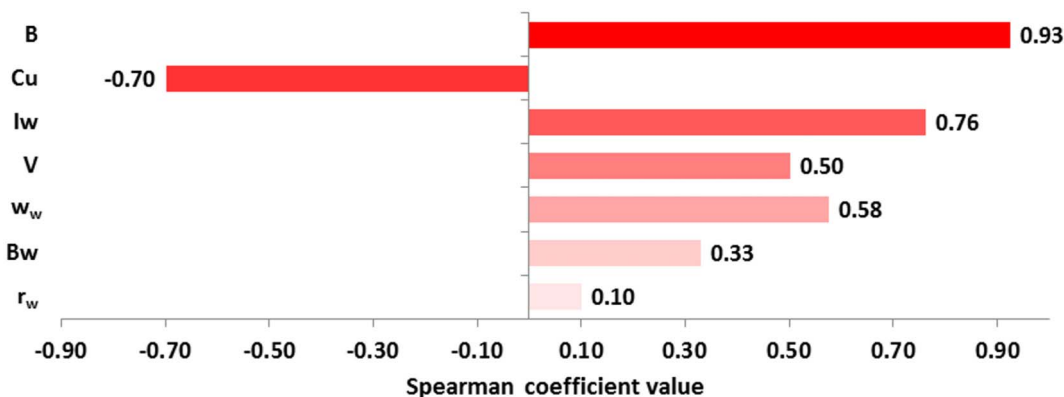
**Cluster analysis.** Spatial, temporal and spatio-temporal cluster analyses were performed using SatScan. Clusters were calculated based on number of cases occurring in an outbreak and susceptible population using a discrete Poisson model<sup>49</sup> (equation 1).

$$S_W = \text{maximum}(W \in W)X(W), \quad (1)$$

Where  $X$  is number of events and  $W$  is the size of the window scan. Subsequently, clusters were mapped in ArcMap (ArcGIS 10.0 ESRI Inc., Redland CA) along with the spatial means of the clusters (World Topographic Map, ArcGIS 10.0 ESRI Inc., Redland CA.; 1970 Datum, Stereographic 70 Projection).

**Estimation of basic reproductive number.** The basic reproductive number ( $R_0$ ) is the mean number of secondary cases caused by an infectious individual during its entire infectious period when introduced to a fully susceptible population<sup>50</sup>. The epidemiological unit of interest in this study is the village; hence the basic reproductive number was defined as the expected number of secondary outbreaks arising from a single individual village outbreak during the entire follow up period.  $R_0$  was calculated by SI modeling method, nearest infectious neighbor method, epidemic doubling time method. Details of the methods used are described in similar previous studies<sup>27</sup>.

**Stochastic mathematical model.** Wild migratory birds are asymptomatic carriers of the virus and may infect local wild birds or poultry at their wintering grounds. Different hypotheses have been put forth, explaining the mechanism of wild migratory birds carrying the virus over long distances<sup>49,51</sup>. In contrast to previous models which considered wild birds to be a one-time infectious source which is specified as a single signal in the model, the current study assumed that wild birds can continuously interact and have contact with poultry at a specific rate. Hence, wild birds could be a continuous source of infection to other wild birds and poultry. We constructed a stochastic continuous-time mathematical model based on frequency dependent transmission, which assumed that the number of adequate contacts was independent of number of poultry or wild bird congregations (Figure 3). Frequency dependent transmission was justified by that a village was considered infected if exposed to adequate contact for transmission of HPAI from a single or several infected wild birds. The domestic-wild bird transmission model design was a



**Figure 2 | Correlation between model inputs and number of infected villages at the peak of the outbreak predicted by a continuous-time stochastic SIC-SIRS mathematical model for Highly Pathogenic Avian Influenza H5N1 transmission in wild birds and poultry in West Bengal, India.** B= Transmission Factor in poultry; Cu= Culling rate in poultry (per day); Iw= Number of Initial wild bird congregations infected with H5N1; V= Proportion of wild birds sharing habitat and having some kind of direct contact with poultry communities; ww= Rate of waning immunity in wild birds after recovery from H5N1 (per day); Bw= Transmission factor in wild birds; rw= Recovery rate in wild birds upon infection with H5N1 (per day).



**Table 4 | Results of a logistic regression model for the association between risk factors and Highly Pathogenic Avian Influenza H5N1 outbreaks in poultry in West Bengal, India during the period between 2008 to 2010**

Variable	Odds Ratio	Standard error	P value	95% confidence limits	
				Lower	Upper
<b>Intercept</b>	0.37	0.61	0.11	0.11	1.22
<b>Sharing habitat with wild birds</b>	5.14	0.57	0.01	1.68	15.76
<b>History of outbreak in district</b>	3.49	0.58	0.03	1.12	10.87
<b>Euclidian distance of outbreak from nearest observed wildlife congregation (km)</b>	0.99	0.01	0.16	0.98	1.01

SIC-SIRS model for the change in disease status of a poultry flock in a village from susceptible (S), infected (I) and culled (C) and wild birds in a wild bird congregation from S, I, recovered (R) and S again.

Each village was considered to have both poultry and wild bird groups susceptible to HPAI infection. Once a flock was infected in a village, it was assumed that the entire village was infected and will be culled. Model assumptions included no decrease in the population of wild birds as evidence suggests the mortality due to HPAI is likely to be minimal<sup>9,52–54</sup>. The proportion of poultry communities having possible connection by sharing the same water-body or via bridge avian species (syn-anthropic wild bird species) among the wild bird congregations were estimated as a stochastic parameter (V) due to inherent variability in its value. The transmission rate between an infected poultry community to a susceptible one ( $\beta$ ) was derived from the  $R_0$  estimated using SI method based on the field data. In contrast, the transmission rate between wild birds and from wild birds to poultry communities ( $\beta_w$ ) was based on a previous study of avian influenza dynamics in wild birds<sup>17</sup>. The following are the ordinary differential equations used for the continuous time-stochastic mathematical model for transmission of HPAI in wild birds and poultry in West Bengal India:

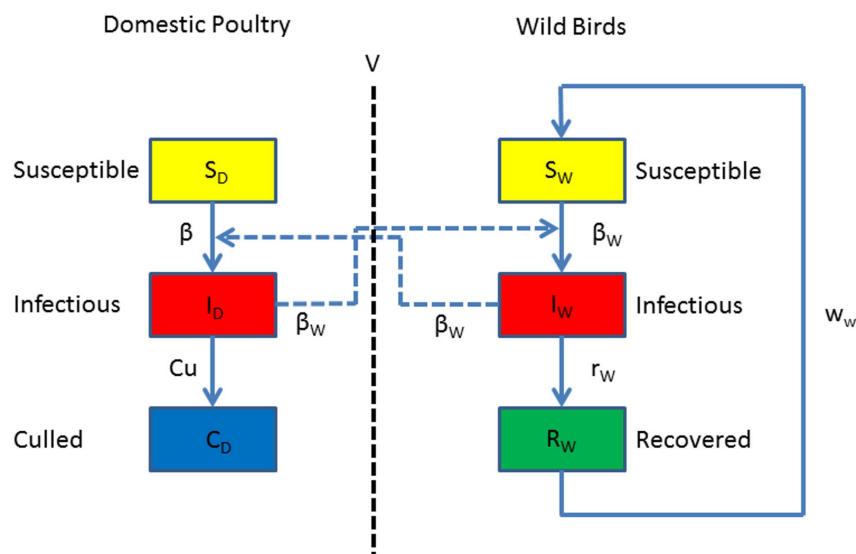
$$\begin{aligned}\frac{dS_D}{dt} &= -\beta S_D I_D - V \beta_w I_W S_D \\ \frac{dI_D}{dt} &= \beta S_D I_D - C u I_D + V \beta_w I_W S_D \\ \frac{dC_D}{dt} &= C u I_D \\ \frac{dS_W}{dt} &= -\beta_w S_W I_W - V \beta_w I_D S_W + w_w R_W \\ \frac{dI_W}{dt} &= \beta_w S_W I_W + V \beta_w I_D S_W - r_w I_W \\ \frac{dR_W}{dt} &= r_w I_W - w_w R_W,\end{aligned}$$

where:

- $S_D$  = Proportion of susceptible villages with domestic poultry
- $I_D$  = Proportion of infected villages with domestic poultry
- $C_D$  = Proportion of villages with culled poultry
- $S_W$  = Proportion of susceptible wild bird congregations
- $I_W$  = Proportion of infected wild bird congregations
- $R_W$  = Proportion of recovered wild birds
- $\beta$  = Transmission factor for transmission of H5N1 between domestic poultry communities
- $\beta_w$  = Transmission factor for transmission of H5N1 from wild birds to domestic poultry communities, and between wild birds
- $V$  = Proportion of wild birds in all forms of sufficient contact with domestic poultry
- $Cu$  = Culling rate of infected domestic poultry
- $r_w$  = recovery rate of infected wild birds
- $w_w$  = Rate at which natural immunity wanes in wild birds

**Estimation of stochastic model parameters.** The model assumed all the villages ( $n=37,945$ ) in the state of West Bengal to be susceptible poultry communities along with an estimated equal number of wild bird congregations<sup>55</sup>. The outbreak started with the introduction of a single infected community of wild birds, hence  $I_W(t=1)$  was estimated at  $1/37,945$ , while  $I_D(t=1)$  was zero. The  $\beta$  estimate based on the SI model of observed data was found to be exponentially distributed ( $\mu = 0.122691$ ). The  $\beta_w$  was estimated at  $1.00E-08$ <sup>17</sup>. Infected poultry were either completely culled or dead within 7 days of the infection and hence a fixed cull rate of  $1/7$  was assumed for the entire study period. Waning immunity in infected wild birds was estimated to be  $0.038/\text{day}$  based on a previous study<sup>17</sup>. During the survey of wild birds in West Bengal it was observed that almost half of the wild bird congregations have contact with poultry by sharing the same habitat or by presence of bridge species. Hence  $V$ , an estimate of the adequate contact between wild birds and poultry was determined to be  $0.5$  and introduced into the model as a stochastic parameter that is normally distributed ( $SD=0.1$ ). Recovery rate of infected wild birds ( $r_w$ ) was considered to be  $17 \text{ days}^{-1}$ . The  $R_0$  was estimated using the total infected villages at the end of the outbreak<sup>56</sup>.

A sensitivity analysis was conducted to investigate the effect of different model inputs on number of infected villages and rank inputs by importance. The Spearman



**Figure 3 | Highly Pathogenic Avian Influenza H5N1 Disease Dynamics:** Infection in poultry communities is described by a SIC model, and in wild bird communities is by a SIRS model. Interactions between poultry and wild birds are depicted as dotted lines. HPAI transmission is governed by the probabilities depicted at every transmission.



Rank correlation coefficient was used to estimate the correlation between each of the model's stochastic inputs and the number of infected villages at the peak of the outbreak. The probability of no outbreak (total infected villages < than 1),  $R_0$  and the number of villages infected at the peak of the outbreak were recorded to investigate the effect of varying the model inputs on these outcomes.

**Risk factor analysis.** Risk factors influencing the incidence of HPAI are well known<sup>44</sup> hence the current study focused on risk factors related to wild birds which might affect the incidence of disease in poultry. Specifically, sightings of birds of Orders Galliformes and Charadriiformes which are considered sentinel species and syn-anthropic wild birds which serve as bridge species<sup>44</sup> in the vicinity of poultry HPAI outbreaks were investigated. Remaining risk factors studied related to the critical community size (CCS) which is defined as the number of individuals able to maintain infection in an environment for certain period of time. The CCS for HPAI was estimated to be 1200–1500 birds which considering the habitat in which wild birds are found, large water-bodies may play a very important role in maintaining the infection. For example, presence or proximity to rice fields is known to be an important risk factor<sup>30</sup>. Other factors investigated are shared habitats between domestic ducks allowed to graze in water bodies with migratory birds, a common practice in places in West Bengal. Hence for the risk factor analysis CCS, habitat of the observed congregation<sup>37</sup>, presence of previously known HPAI infected species<sup>58</sup>, sentinel species and bridge species in the avian community of the congregation and presence of domestic ducks in the same habitat<sup>59</sup> were considered.

Fifty-four random points were generated in the map of West Bengal as controls. Nearest observed wild bird community was used as predictors for both outbreaks and controls. Logistic regression was used to study the association between the various putative risk factors (mentioned before in the paragraph above) and the presence of HPAI infection. The association was estimated as the ratio of the odds of exposure to a risk factor in a village with H5N1 infected poultry to the odds of exposure to that risk factor in an uninfected village. A forward manual step-wise model building algorithm was implemented with a two-sided P value  $\leq 0.10$  for the inclusion of a risk factor in the model was used. Competing models were compared using the likelihood ratio test. The final model's goodness of fit was assessed using the deviance with a chi square test. Confounding of the association between sharing of habitat by poultry and wild birds and presence of a H5N1 outbreak in poultry by each of the risk factors was assessed using the method of change in estimates. The model was adjusted for a confounder if inclusion of the variable changed the estimated ORs by more than 10%<sup>60</sup>.

The logistic regression model was a generalized linear model with a logit link. The model predicted the log odds of the probability of an H5N1 outbreak in the  $i^{\text{th}}$  domestic flock as predicted by putative risk factors and was formulated as below:

$$\text{Logit}(p_i) = \beta_0 + \beta_{\text{Shared Habitat}} X_{i,\text{Shared Habitat}} + \beta_{\text{Sentinel Species}} X_{i,\text{Sentinel Species}} + \beta_{\text{Bridge Species}} X_{i,\text{Bridge Species}} + \beta_{\text{Previously infected Species}} X_{i,\text{Previously infected Species}} + \beta_{\text{Habitat}} X_{i,\text{Habitat}} + \beta_{\text{Critical community size}} X_{i,\text{Critical community size}} + \beta_{\text{Outbreak History}} X_{i,\text{Outbreak History}} + \beta_{\text{Distance to nearest observed wildlife}} X_{i,\text{Distance to nearest observed wildlife}}$$

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## Author contributions

Risk factor data collection: PP, SP. Conceived and designed mathematical model: PP, SA. Statistical and spatial analysis: PP, SA. Biological input on mathematical and statistical models: DB. Wrote first draft of the manuscript: PP. Wrote and critically reviewed the final manuscript: DB, SP, SA.

## Additional information

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